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Synthesis of Milbemycins β_9 and β_{10} from Milbemycins A_3 and A_4 and Their Biological Activities

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Note

Synthesis of Milbemycins β_9 and β_{10} from Milbemycins A_3 and A_4 and Their Biological Activities

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Chemical derivation methods were used to prepare milbemycins β_9 and β_{10} from milbemycins A_3 and A_4. Their acaricidal activities were also assessed against the organophosphorus-sensitive two-spotted spider mite (*Tetranychus urticae*) on primary leaves of cowpea plants (*Vigna sinesis Savi* species) by spraying.

Key words: milbemycin; acaricide

Milbemycins[1–2] are sixteen-membered ring macrolides that have been isolated from *Streptomyces hygroscopicus* subsp. *aureolacrimosus*. They exhibit notable activities as acaricides, insecticides and anthelmintics. Among them, milbemectin[3] [a mixture of milbemycins A_3 (**1**) and A_4 (**2**) (Fig. 1)] has been developed as an agricultural acaricide. Since the discovery of milbemycins, numerous homologues such as Merck's avermectins[4] and Cyanamid's LL-F28249 series (nemadectins)[5] have been isolated and reported. Enormous efforts have been made to study the biosynthetic pathways to milbemycins,[6] and many congeners, which have the same sixteen-membered ring moiety, have consequently been isolated. In a recent study on the biosynthetic pathways to milbemycins, Nonaka *et al.* have reported the discovery of milbemycins β_9 (**3**) and β_{10} (**4**) (Fig. 1).[6c] Interest in their biological activities and their necessity as reference materials for further study of the biosynthetic pathways required preparing them by chemical derivation from milbemycins A_3 (**1**) and A_4 (**2**), because of their low biosynthetic productivity.[6c] We report in this paper the chemical derivations from milbemycins A_3 (**1**) and A_4 (**2**) of milbemycins β_9 (**3**) and β_{10} (**4**), and their acaricidal activities.

The straightforward chemical derivation from milbemycins A_3 (**1**) and A_4 (**2**) of milbemycins β_9 (**3**) and β_{10} (**4**) was achieved as shown in the Figure. Milbemycin A_3 (**1**) was methylated as reported[7] to afford 5-*O*-methylated derivative **5** (85.9%), together with 5-*O*, 7-*O*-dimethylated derivative **7** (3.8%) as a by-product. In the case of milbemycin A_4 (**2**), the yield of **6** decreased to 39.9% due to the increased formation of 5-*O*, 7-*O*-dimethylated by-product **8** (22.7%). These results may have been due to the difference in solubility of milbemycins A_3 (**1**) and A_4 (**2**) in acetonitrile. In the reaction, milbemycin A_3 (**1**) was suspended in acetonitrile, but milbemycin A_4 (**2**) was completely dissolved. After purification by preparative HPLC, 5-*O*-methylated derivatives **5** and **6** were oxidized to 27-oxo derivatives **9** and **10** with chromium (VI) oxide (CrO_3)[8] in 15.6% and 24.4% yields, respectively. Considerable amounts of the over-oxidized products, 5-*O*-formyl-27-oxo derivative **11** (5.5%) and **12** (6.5%), were formed in this reaction, and residual **5** and **6** were degraded into messy products. Reduction of the 27-lactone moiety of **9** and **10** with diisobutylaluminum hydride (DIBAL-H) provided milbemycins β_9 (**3**) (57.6%) and β_{10} (**4**) (49.6%). All spectral data for **3** and **4** synthesized here are in agreement with those of the reference materials.[6c]

The acaricidal activities of milbemycins β_9 (**3**) and β_{10} (**4**) were studied by spraying on primary leaves of cowpea plants (*Vigna sinesis Savi* species) infested with the organophosphorus-sensitive two-spotted spider mites (*Tetranychus urticae*). The results are summarized in the Table 1 Parent milbemycins A_3 (**1**) and A_4 (**2**) exhibited high acaricidal activities, while milbemycins β_9 (**3**) and β_{10} (**4**) exhibited only poor acaricidal activities.

Experimental

NMR spectra were measured with a Varian Gemini-200 FT NMR spectrometer (200 MHz) or a Jeol JNM-GX-270 FT NMR spectrometer (270 MHz). Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane. Mass spectra were measured by a Fisions Instruments VG Autospec, and IR spectra were measured by a Perkin Elmer 1600 series FT IR instrument.

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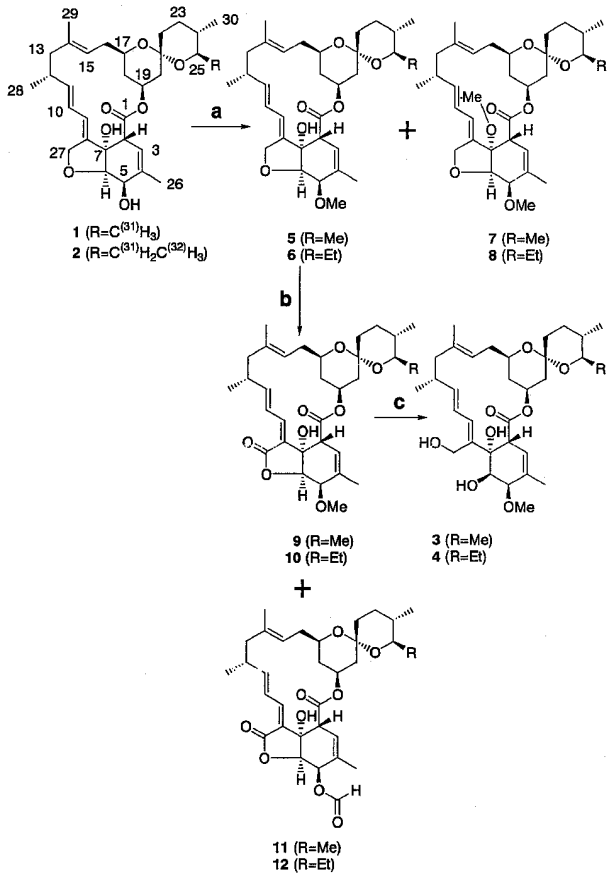

Fig. 1. Structure of Milbemycins and Reaction Conditions. Milbemycins A_3 (**1**), A_4 (**2**), β_9 (**3**) and β_{10} (**4**); a) MeI, Ag_2O, acetonitrile; b) CrO_3, pyridine; c) DIBAL-H, toluene-THF

Methylation of milbemycin A_3 (1) with methyl iodide (MeI) and silver (I) oxide (Ag_2O). To a stirred suspension of **1** (1.00 g, 1.89 mmol) in acetonitrile (20 ml) at ambient temperature were added MeI (0.71 ml, 11.36 mmol) and Ag_2O (878.3 mg, 3.79 mmol). After stirring overnight, the reaction mixture was filtered with Celite,® and the resulting filtrate was evaporated *in vacuo*. The residue was purified by silica gel column chromatography [*n*-hexane (Hex)-ethyl acetate (EtOAc) gradient] and preparative HPLC (YMC-Pack ODS S-365-10, I-10 μm 120A, 30 mm I.D. × 500 mm, acetonitrile-water) to give 881.7 mg (85.9%) of **5** and 3.8 mg (3.8%) of **7** as colorless amorphous solids.

5-O-Methylmilbemycin A_3 (5): IR ν_{max} (film) cm^{-1}: 3465, 2960, 2915, 2860, 1730, 1710; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.67–5.81(2H, m, H-9, H-10), 5.29–5.44(3H, m, H-3, H-11, H-19), 4.98(1H, m, H-15), 4.71(1H, d, J=14.7 Hz, H-27), 4.61(1H, d, J=14.7 Hz, H-27), 4.15(1H, s, 7-OH), 4.03(1H, d, J=5.5 Hz, H-6), 3.96(1H, m, H-5), 3.40–3.65(1H, m, H-17), 3.51(3H, s, CH_3O), 3.20–3.35(2H, m, H-2, H-25), 2.35–2.50(1H, m, H-12), 2.10–2.30(3H, m, H-13, H_2-16), 1.82(3H, br, H_3-26), 1.62(3H, br, H_3-29), 1.14(3H, d, J=6.6Hz, H_3-28), 1.00(3H, d, J=6.6 Hz, H_3-31), 0.83(3H, d, J=6.6 Hz, H_3-30), 0.82–2.05(10H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 542 (M^+), 492; HREI-MS (*m/z*): [M^+]: calcd. for $C_{32}H_{46}O_7$, 542.3244; found, 542.3243.

Table 1. Acaricidal Activity of Milbemycins against *Tetranychus urticae*

	Mortality (%)	
	10 ppm	1 ppm
Milbemycin β_9 (**3**)	10	0
Milbemycin β_{10} (**4**)	0	0
Milbemycin A_3 (**1**)	69	3
Milbemycin A_4 (**2**)	100	32

5-O, 7-O-Dimethylmilbemycin A_3 (7): IR ν_{max} (film) cm^{-1}: 2960, 2915, 2860, 1730; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.66–5.90(3H, m, H-3, H-9, H-10), 5.38(1H, dd, J=14.3, 9.9 Hz, H-11), 4.95–5.20(2H, m, H-15, H-19), 4.67(1H, dd, J=13.9, 2.2 Hz, H-27), 4.57(1H, dd, J=13.9, 1.8 Hz, H-27), 4.27(1H, d, J=5.5 Hz, H-6), 3.94(1H, m, H-5), 3.51–3.55(1H, m, H-17), 3.48(3H, s, CH_3O), 3.38(1H, dd, J=4.8, 2.2 Hz, H-2), 3.19–3.30(1H, m, H-25), 3.27(3H, s, CH_3O), 2.40–2.60(1H, m, H-12), 2.10–2.32(3H, m, H-13, H_2-16), 1.83(3H, br, H_3-26), 1.55(3H, s, H_3-29), 1.14(3H, d, J=6.2 Hz, H_3-28), 1.03(3H, d, J=6.6 Hz, H_3-31), 0.82(3H, d, J=6.6 Hz, H_3-30), 0.73–2.00(10H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 556 (M^+), 492; HREI-MS (*m/z*): [M^+]: calcd. for $C_{33}H_{48}O_7$, 556.3400; found, 556.3401.

Methylation of milbemycin A_4 (2) with MeI and Ag_2O. Using the same procedure as that described for the preparation of **5**, milbemycin A_4 (**2**) (10.00 g, 18.5 mmol) was methylated with MeI (6.9 ml, 110.7 mmol) and Ag_2O (8.5 g, 36.9 mmol) to give 4.10 g (39.9%) of **6** and 2.39 g (22.7%) of **8** as colorless amorphous solids.

5-O-Methylmilbemycin A_4 (6): IR ν_{max} (film) cm^{-1}: 3440, 2960, 2915, 2870, 1730, 1705; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.67–5.82(2H, m, H-9, H-10), 5.29–5.43(3H, m, H-3, H-11, H-19), 4.96(1H, m, H-15), 4.71(1H, d, J=14.9 Hz, H-27), 4.62(1H, d, J=14.9 Hz, H-27), 4.16(1H, s, 7-OH), 4.03(1H, d, J=5.7 Hz, H-6), 3.95(1H, m, H-5), 3.45–3.65(1H, m, H-17), 3.51(3H, s, CH_3O), 3.30(1H, dd, J=4.8, 2.5 Hz, H-2), 3.07(1H, dt, J_t=9.2 Hz, J_d=2.6 Hz, H-25), 2.30–2.50(1H, m, H-12), 2.10–2.30(3H, m, H-13, H_2-16), 2.00(1H, m, H-20), 1.81(3H, br, H_3-26), 1.53(3H, br, H_3-29), 1.00(3H, d, J=6.5 Hz, H_3-28), 0.98(3H, t, J=6.5 Hz, H_3-32), 0.82(3H, d, J=6.3 Hz, H_3-30), 0.75–1.90(11H, m, H-13, H_2-18,

H-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 556 (M^+), 506; HREI-MS (*m/z*): [M^+]: calcd. for $C_{33}H_{48}O_7$, 556.3400; found, 556.3399.

5-O, 7-O-Dimethylmilbemycin A_4 (8): IR ν_{max} (film) cm^{-1}: 2960, 2915, 2870, 1730; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 5.67–5.91(3H, m, H-3, H-9, H-10), 5.40(1H, dd, J=13.9, 9.5 Hz, H-11), 4.96–5.13(2H, m, H-15, H-19), 4.65(2H, m, H_2-27), 4.27(1H, d, J=5.1 Hz, H-6), 3.94(1H, d, J=5.1 Hz, H-5), 3.60(1H, m, H-17), 3.48(3H, s, CH_3O), 3.39(1H, m, H-2), 3.28(3H, s, CH_3O), 3.05(1H, m, H-25), 2.40–2.60(1H, m, H-12), 2.10–2.35(3H, m, H-13, H_2-16), 1.83(3H, br, H_3-26), 1.55(3H, br, H_3-29), 1.03(3H, d, J=6.6 Hz, H_3-28), 1.01(3H, t, J=6.2 Hz, H_3-32), 0.81(3H, d, J=6.6 Hz, H_3-30), 0.79–1.95(12H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 570 (M^+), 506; HREI-MS (*m/z*): [M^+]: calcd. for $C_{34}H_{50}O_7$, 570.3557; found, 570.3554.

*Oxidation of **5** with CrO_3 in pyridine.* To stirred pyridine (20 ml) in an ice bath was slowly added CrO_3 (369.0 mg, 3.69 mmol) while maintaining the temperature at under 10°C. To this solution was added a solution of 5-*O*-methyl-milbemycin A_3 (**5**; 200.0 mg, 0.37 mmol) in pyridine (2 ml). After stirring for 1 hour, the ice bath was removed, and the mixture stirred at ambient temperature. After stirring for 6 days, the reaction mixture was poured into a mixture of 1 N hydrochloric acid and EtOAc. After stirring for 15 min, the insoluble material was filtered off with Celite, ® and the filtrate was extracted with EtOAc. The extract was successively washed with 1 N hydrochloric acid, water and brine, dried over magnesium sulfate ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (Hex-EtOAc gradient) and preparative HPLC (YMC-Pack ODS S-365-10, I-10 μm 120A, 30 mm I.D. × 500 mm, acetonitrile-water) to give 32.0 mg (15.6%) of **9** and 11.6 mg (5.5%) of **11** as colorless amorphous solids.

5-O-Methyl-27-oxomilbemycin A_3 (9): IR ν_{max} (film) cm^{-1}: 3465, 2960, 2915, 2870, 1755, 1730, 1710, 1640; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.30(1H, dd, J=15.0, 11.4 Hz, H-10), 6.53(1H, d, J=11.4 Hz, H-9), 5.85(1H, dd, J=15.0, 9.9 Hz, H-11), 5.70(1H, br, H-3), 5.50(1H, m, H-19), 5.03(1H, m, H-15), 4.69(1H, s, 7-OH), 4.35(1H, d, J=6.5 Hz, H-6), 4.02(1H, m, H-5), 3.65(1H, dd, J=5.5, 2.9 Hz, H-2), 3.50–3.65(1H, m, H-17), 3.43(3H, s, CH_3O), 3.28(1H, d, J=9.5, 6.2 Hz, H-25), 2.55–2.75(1H, m, H-12), 2.15–2.35(3H, m, H-13, H_2-16), 1.93(3H, br, H_3-26), 1.53(3H, br, H_3-29), 1.15(3H, d, J=6.2 Hz, H_3-28), 1.03(3H, d, J=6.6 Hz, H_3-31), 0.84(3H, d, J=6.6 Hz, H_3-30), 0.82–2.05(10H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 556 (M^+), 538, 506; HREI-MS (*m/z*): [M^+]: calcd. for $C_{32}H_{44}O_8$, 556.3036; found, 556.3036.

5-O-Formyl-27-oxomilbemicin A_3 (11): IR ν_{max} (film) cm^{-1}: 3450, 2960, 2915, 2870, 1755, 1725, 1640; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 8.09(1H, s, OCHO), 7.27(1H, dd, J=15.4, 11.4 Hz, H-10), 6.55(1H, d, J=11.4 Hz, H-9), 5.89(1H, dd, J=15.4, 9.9 Hz, H-11), 5.79(1H, br, H-3), 5.70(1H, d, J=6.2 Hz, H-5), 5.50(1H, m, H-19), 5.03(1H, m, H-15), 4.70(1H, s, 7-OH), 4.45(1H, d, J=6.2 Hz, H-6), 3.55–3.70(1H, m, H-17), 3.50(1H, dd, J=5.1, 2.6 Hz, H-2), 3.28(1H, dd, J=9.5, 6.2 Hz, H-25), 2.55–2.70(1H, m, H-12), 2.15–2.40(3H, m, H-13, H_2-16), 1.94(3H, br, H_3-26), 1.54(3H, br, H_3-29), 1.15(3H, d, J=6.2 Hz, H_3-31), 1.04(3H, d, J=6.6 Hz, H_3-28), 0.84(3H, d, J=6.2 Hz, H_3-30), 0.83–2.05(10H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24); EI-MS (*m/z*): 570 (M^+); HREI-MS (*m/z*): [M^+]: calcd. for $C_{32}H_{42}O_9$, 570.2829; found, 570.2831.

*Oxidation of **6** with CrO_3.* Using the same procedure as that described for the preparation of **9**, **6** (3.00 g, 5.40 mmol) was oxidized with CrO_3 (4.69 g, 46.94 mmol) in pyridine (330 ml) to give 0.75 g (24.4%) of **10** and 0.20 g (6.5%) of **12** as colorless amorphous solids.

5-O-Methyl-27-oxomilbemicin A_4 (10): IR ν_{max} (film) cm^{-1}: 3475, 2960, 2915, 2870, 1750, 1735, 1710, 1640; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 7.28(1H, dd, J=15.0, 11.2 Hz, H-10), 6.53(1H, d, J=11.2 Hz, H-9), 5.87(1H, dd, J=15.0, 9.6 Hz, H-11), 5.69(1H, br, H-3), 5.40–5.55(1H, m, H-19), 4.99(1H, m, H-15), 4.67(1H, s, 7-OH), 4.34(1H, d, J=5.5 Hz, H-6), 4.02(1H, d, J=5.5 Hz, H-5), 3.50–3.65(2H, m, H-2, H-17), 3.42(3H, s, CH_3O), 3.07(1H, dt, J_t=9.2 Hz, J_d=2.3 Hz, H-25), 2.50–2.70(1H, m, H-12), 2.15–2.30(3H, m, H-13, H_2-16), 1.92(3H, br, H_3-26), 1.52(3H, br, H_3-29), 1.02(3H, d, J=6.9 Hz, H_3-28), 0.99(3H, t, J=6.9 Hz, H_3-32), 0.82(3H, d, J=6.4 Hz, H_3-30), 0.80–2.05(12H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (*m/z*): 570 (M^+), 552, 520; HREI-MS (*m/z*): [M^+]: calcd. for $C_{33}H_{46}O_8$, 570.3193; found, 570.3192.

5-O-Formyl-27-oxomilbemicin A_4 (12): IR ν_{max} (film) cm^{-1}: 3455, 2960, 2915, 2870, 1755, 1730, 1640; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 8.09(1H, s, OCHO), 7.25(1H, dd, J=15.1, 11.4 Hz, H-10), 6.56(1H, d, J=11.4 Hz, H-9), 5.91(1H, dd, J=15.1, 10.1 Hz, H-11), 5.78(1H, br, H-3), 5.70(1H, d, J=6.3 Hz, H-5), 5.40–5.60(1H, m, H-19), 5.00(1H, m, H-15), 4.70(1H, s, 7-OH), 4.45(1H, d, J=6.3 Hz, H-6), 3.50–3.70(1H, m, H-17), 3.49(1H, m, H-2),

3.08(1H, dt, J_t=9.1 Hz, J_d=2.4 Hz, H-25), 2.55–2.80(1H, m, H-12), 2.20–2.35(3H, m, H-13, H_2-16), 1.93(3H, d, J=1.9 Hz, H_3-26), 1.53(3H, s, H_3-29), 1.03(3H, d, J=6.7 Hz, H_3-28), 1.00(3H, t, J=7.3 Hz, H_3-32), 0.83(3H, d, J=6.3 Hz, H_3-30), 0.82–2.05(12H, m, H-13, H_2-18, H_2-20, H_2-22, H_2-23, H-24, H_2-31); EI-MS (m/z): 584 (M^+), 520; HREI-MS (m/z): [M^+]: calcd. for $C_{33}H_{44}O_9$, 584.2985; found, 584.2987.

*Reduction of **9** with DIBAL-H.* To a stirred solution of **9** (30 mg, 0.05 mmol) in toluene (1 ml) was added dropwise a 1 M solution of DIBAL-H in tetrahydrofuran (THF; 0.16 ml, 0.16 mmol) while cooling in an ice bath. After stirring for 30 min in the ice bath, an additional 1 M solution of DIBAL-H in THF (0.16 ml, 0.16 mmol) was added dropwise. After stirring for 40 min more in the ice bath, an additional 1 M solution of DIBAL-H in THF (0.32 ml, 0.32 mmol) was again added dropwise. After stirring for a final 40 min in the ice bath, the reaction mixture was poured into 1 N hydrochloric acid and extracted with EtOAc. The extract was successively washed with water and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by preparative TLC (Hex-EtOAc=1:2) to give 17.4 mg (57.6%) of milbemycin β_9 (**3**) as a colorless amorphous solid.

*Reduction of **10** with DIBAL-H.* Using the same procedure as that described for the preparation of milbemycin β_9 (**3**), **10** (200.0 mg, 0.35 mmol) was reduced with DIBAL-H in toluene to give 99.9 mg (49.6%) of milbemycin β_{10} (**4**) as a colorless amorphous solid.

Acaricidal activity against Tetranychus urticae. The primary leaves of cowpea plants (*Vigna sinensis Savi* species) were infected with the organophosphorus-sensitive two-spotted spider mites (*Tetranychus urticae*). One day after infection, the infested plants were sprayed by a Mizuho rotary sprayer with 7 ml of a solution containing the test compound at a concentration ranging from 1 to 10 ppm at a rate of 3.5 mg of the test solution per 1 cm^2 of leaf. The plants were assessed after 3 days by examining the adult mites under a binocular microscope to determine the numbers of living and dead individuals. Two plants were used for each concentration and each test compound. The plants were kept during the test in green-house compartments at 25°C.

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